Analysis of Residual Acrylamide In Field Crops

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Abstract

Polyacrylamide (PAM) is a widely used product for a large number of applications. Many of the emerging applications are in the area of agriculture. PAM is blended with pesticides as a thickening agent, added to irrigation water to minimize soil erosion, and used as a medium for hydroponically grown crops. Although PAM is stable and considered to be safe, residual acrylamide (AMD) monomer is a neurotoxin and animal carcinogen. In this work, residual AMD is analyzed in a variety of crops that were grown under PAM treatment to stabilize soil erosion. Corn, potatoes, sugar beets, and beans are analyzed for AMD. A sample of the crop is homogenized with water, and the water layer is filtered and derivatized with bromine to form 2,3-dibromopropionamide. The derivative is then extracted with ethyl acetate and converted to the more stable 2-propenamide prior to gas chromatographic analysis using an electron capture detector. Capillary Carbowax columns were used. All tested crops show < 10 ppb AMD. Furthermore, it seems that AMD is not stable when it comes in contact with the crop tissues. In the presence of plant tissues, AMD will dissapear as a function of time. Beans blended with 100 ppb AMD for 10 min yield a recovery of only 22%. For a bean sample that was soaked with 500 ppb AMD solution for 18 h, the recovery is 7%. Other crops show different AMD recoveries.

Introduction

Polyacrylamide (PAM) and copolymers of PAM are made with a high molecular weight of up to 10–20 million g/mole. The polymer can be made anionic, nonionic, or cationic. Polymers of different molecular weight are employed in a wide range of applications, include water and waste water clarification, biosolids dewatering, paper making, mineral dispersion, food processing for humans and animals, petroleum production, cosmetics, textile production, and many agricultural applications that minimize pesticide drift and control soil erosion (1). Highly crosslinked water-swellable PAMs are used to enhance water retention in horticulture, for example.

Although PAMs exhibit a low order of toxicity to mammalian systems, the monomer itself is a neurotoxin and an animal carcinogen (2,3). Several researchers have investigated residual acrylamide (AMD) in agricultural products that utilized PAM (4–6). Others have investigated the fate of PAM in soil (7–9).

The analysis of trace residual AMD in complex mixtures such as field crops is a difficult task because of chromatographic interferences, poor repeatability, and the need to achieve very low levels of detection. Most of the analytical methods reported for the analysis of AMD at trace level are based on derivatization to 2,3-dibromopropionamide from aqueous solution (10) followed by gas chromatographic (GC) analysis using an electron capture detector (ECD) or mass spectrometer (MS). It was found, however, that the 2,3-dibromopropionamide is not stable, and some of the derivative can be converted to the more stable derivative 2-bromopropenamide on the inlet of the GC or on the column (11). Because this conversion will yield poor repeatability and accuracy, the 2,3-dibromopropionamide derivative is converted to the stable 2-bromopropenamide prior to GC analysis by adding a small amount of triethylamine (11).

Arikawa et al. (6) analyzed AMD in 10 field crops. They reported less than 5 ppb in all the crops they tested using GC–ECD. The authors also reported recoveries of 80–98%, but they spiked the extracted, filtered solution of the sample. In this case, the sample tissues did not come in contact with the spiked AMD. Castle et al. (4) analyzed AMD in tomato fruits grown hydroponically in PAM solution. The PAM solution was further enriched with AMD, and less than 1 ppb was detected. They used internal standards and selected ion monitoring GC–MS, yielding recoveries of 26–62%. Castle (5) used the same procedure to analyze AMD uptake in mushrooms. Castle detected less than 0.5 ppb AMD and concluded that AMD either does not translocate significantly from the mycelia to the mushrooms or does not bioaccumulate because of chemical reactivity.

In the work reported here, PAM is analyzed in 4 different crops (beans, sugar beets, corn, and potatoes) grown in soil treated

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with PAM to reduce soil erosion. A direct method for sample preparation was used as described in the following section. The stability of AMD in the presence of sample tissues for the different crops was studied.

Experimental

Sample preparation

The bean samples were the only crop that was soaked in water overnight prior to homogenizing it for extraction. The corn samples were shaved from the cob with a knife prior to the addition of water. The sugar beet pulp and skin was cut into 3–4 pieces with a knife and grated with a hand-held vegetable grater prior to the addition of water. Potato pulp and potato skin were analyzed separately. The skin was peeled from the potato pulp, and the potato pulp was cut and grated in the same manner as the sugar beet.

To extract AMD from the tissue matrix, 10 g of the crop tissue were homogenized with 50 mL high-performance liquid chromatography (HPLC)-grade water for different periods of time. The aqueous layer was centrifuged for 5 min at 10,000 rpm and/or filtered using a Buchner funnel and vacuum pump.

Derivatization

The filtrate was derivatized in a similar manner as in previous reports (10,11). Potassium bromide (7.5 g) was dissolved with stirring and the pH was adjusted to a value between 1 and 3 by the addition of a few drops of hydrobromic acid. The flask was transferred into an ice bath and covered with aluminum foil. A saturated bromine–water solution (2.5 mL) was added to the flask while stirring. The reaction was allowed to take place for 1 h in an ice bath. After the reaction was completed, the excess bromine was decomposed by the addition of a few drops of 1M sodium thiosulfate solution that removed the yellowish color of the bromine solution. Sodium sulfate (15 g) was added while stirring. The resulting solution was transferred into a 250-mL separatory funnel. The aqueous solution was extracted with 10 mL ethyl acetate by shaking for 2 min. The aqueous layer on the bottom was transferred into the original 250-mL round-bottom flask. The ethyl acetate layer was transferred into a clean 20-mL glass centrifuge tube. The aqueous layer in the flask was transferred into the separatory funnel, and extraction was repeated using a second 10-mL portion of ethyl acetate. The 2 layers were then separated, and the ethyl acetate was combined with the first 10-mL portion. Upon difficulty in separating the layers, the mixtures were centrifuged at 5,000 rpm to achieve a good separation. The organic phase was dried with 1 g sodium sulfate. A 1.5-mL aliquot of the extract was transferred to an autosampler vial. Prior to capping the vial, 100 µL of triethylamine was added to the extract.

Standard, spiking and recoveries

The standards were prepared as mentioned previously in the derivatization. When spiking the 4 crop samples, the water used for homogenizing contained AMD monomer at different concentrations.

GC conditions

A Hewlett-Packard 5890 GC equipped with an ECD was used. The ECD was operated at 300°C. The nitrogen carrier gas was of ultrahigh-purity (99.999%) grade. The chromatographic signal was recorded and integrated on a Perkin Elmer (Norwalk, CT) Access Chrom chromatography data system. A DBwax (polyethylene glycol) capillary column (30 m × 0.53-mm i.d., 1.0-µm film thickness) was used for this work (J&W Scientific, Folsom, CA). The overhead pressure was 18 psi. The injector was operated at 275°C. The split flow rate of nitrogen was 40 mL/min. The column was operated at 100°C for 1 min and then heated to 170°C for 17 min. To clean up the column, it was heated to 185°C for 5 min after the sample analysis was completed.

Reagents

Ethyl acetate (99.9% pure) was purchased from J.T. Baker (Phillipsburg, NJ). Triethylamine (99% pure) was purchased from Aldrich (Milwaukee, WI). Omnisolv HPLC-grade water was purchased from EM science (Gibbstown, NJ). Acrylamide (> 99%, electrophoresis grade) was obtained from Aldrich. Sodium sulfate (anhydrous) was obtained from Baker Analyzed. Potassium bromide (> 99% pure) was obtained from Aldrich. Bromine ("reagent") was obtained from Baker Analyzed. Hydrobromic acid (47–49% pure) was obtained from Baker Analyzed.

Details about the PAM dosing for irrigation will be published elsewhere (in preparation by Lentz and Sojka).

Results and Discussion

The linearity of response for the derivatized AMD standards was investigated. Standards of 2–100 ppb were derivatized. The response was linear between 5 and 100 ppb with a linearity coefficient r^2 of 0.9990. Below 5 ppb, the response deviates from linearity. The limit of quantitation for this work was considered to be only 10 ppb because of small chromatographic interferences in the samples analyzed. The results are listed in Table I.

The repeatability of 6 replicates of 50-ppb derivatized AMD standards was a relative standard deviation (RSD) of 14%. The repeatabilty of 6 injections of one of the derivatives was RSD = 9%.

All of the samples analyzed for the 4 crops showed less than 10 ppb residual AMD. Figures 1–3 show typical chromatograms.

Table I. Linearity of Response for the Derivatized AMD Standards

Concentration (ppb)	Response factor (area/concentration, ppb)
2	9308
5	5894
10	5341
20	4606
50	4990
100	5142

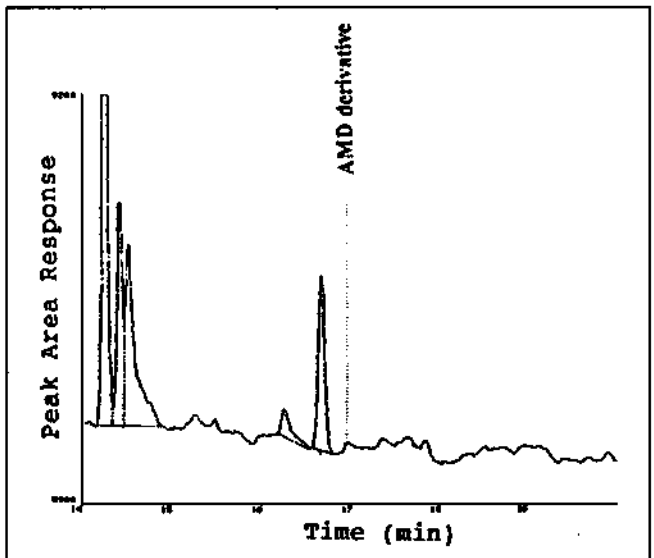

Figure 1. Chromatogram of bean extract.

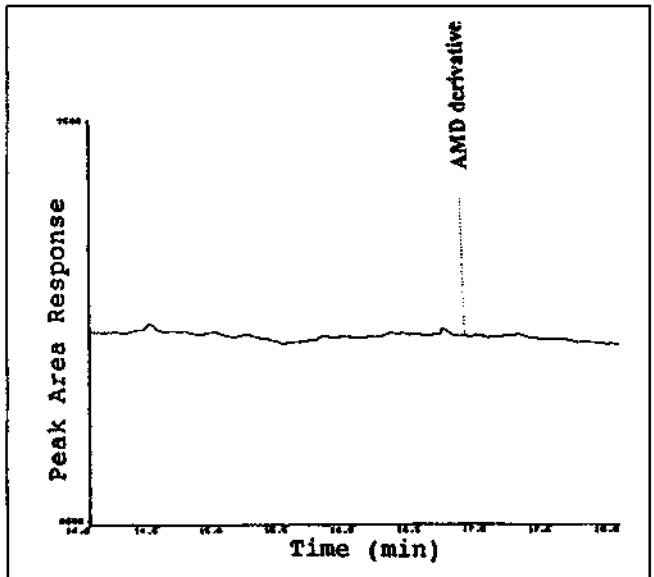

Figure 2. Chromatogram of sugar beet extract.

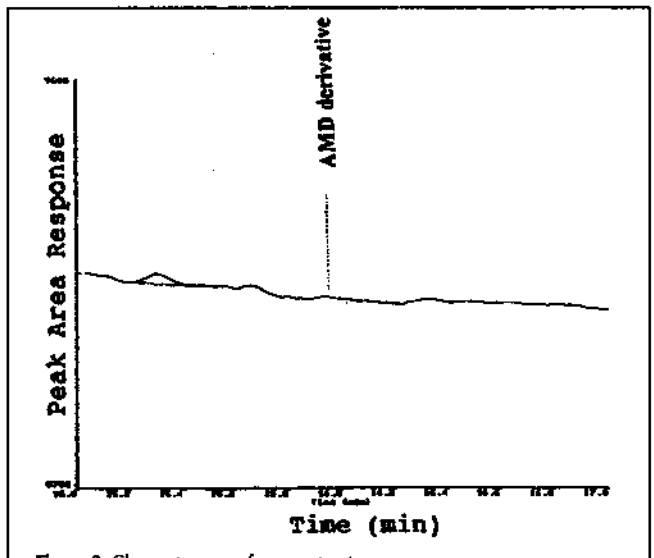

Figure 3. Chromatogram of corn extract.

Although a small peak appeared at the retention time of AMD, the concentration of this peak is « 10 ppb, and it is most likely not attributed to AMD (as will be shown later in the text).

Beans

Six batches of beans (6 different field treatments) were analyzed and found to contain less than 10 ppb AMD. The results for bean samples soaked in water, spiked with 100 ppb AMD, and blended for different periods are shown in Table II. A dry bean sample soaked for 18 h in water containing 500 ppb AMD and then blended for 1 min showed a recovery of only 7%.

Table II. Recovery of AMD from Beans

Blending time	Recovery (%)
10 s	75
1 min	56
2 min	46
5 min	29
10 min	22

Sugar beet

Six sugar beet samples were analyzed. No AMD was detected at any level above 10 ppb. A sample that was spiked with 100 ppb AMD and blended for 1 min showed a recovery of 93%. Another sample spiked with 200 ppb and blended for 5 min showed a recovery of 77%.

Corn

Six batches of fresh corn were analyzed and found to contain less than 10 ppb. The amount of AMD recovered after spiking with 100 ppb AMD was dependent on the contact time with AMD during blending. Figure 4 shows three chromatograms for corn spiked with 50 ppb AMD and blended for different time periods. Results are listed in Table III. Three independent corn samples were spiked with 50, 100, and 200 ppb AMD and blended for 1 min. The recovery of AMD was approximately 100%. It was linear with a correlation coefficient r^2 of 0.9989.

Potatoes

Six batches of potatoes were analyzed. The skin and the pulp were analyzed independently. The detected concentration of AMD was less than 10 ppb, as shown in Figures 5 and 6. When 6 pulp samples were spiked with 100 ppb AMD, the recovery was 83% with an RSD of 21% for 5 replicates. For the skin, the recovery was 104% with an RSD of 34%. There seemed to be no significant difference in AMD recovery when it was blended with

Table III. Recovery of AMD from Corn

Blending time	Recovery (%)
10 s	112
1 min	103
5 min	26

the potatoes for up to 20 min. The results are shown in Table IV. Although AMD was not detected in any of the 6 batches tested, it seems that AMD is more stable in the potatoes than in the rest of the crops.

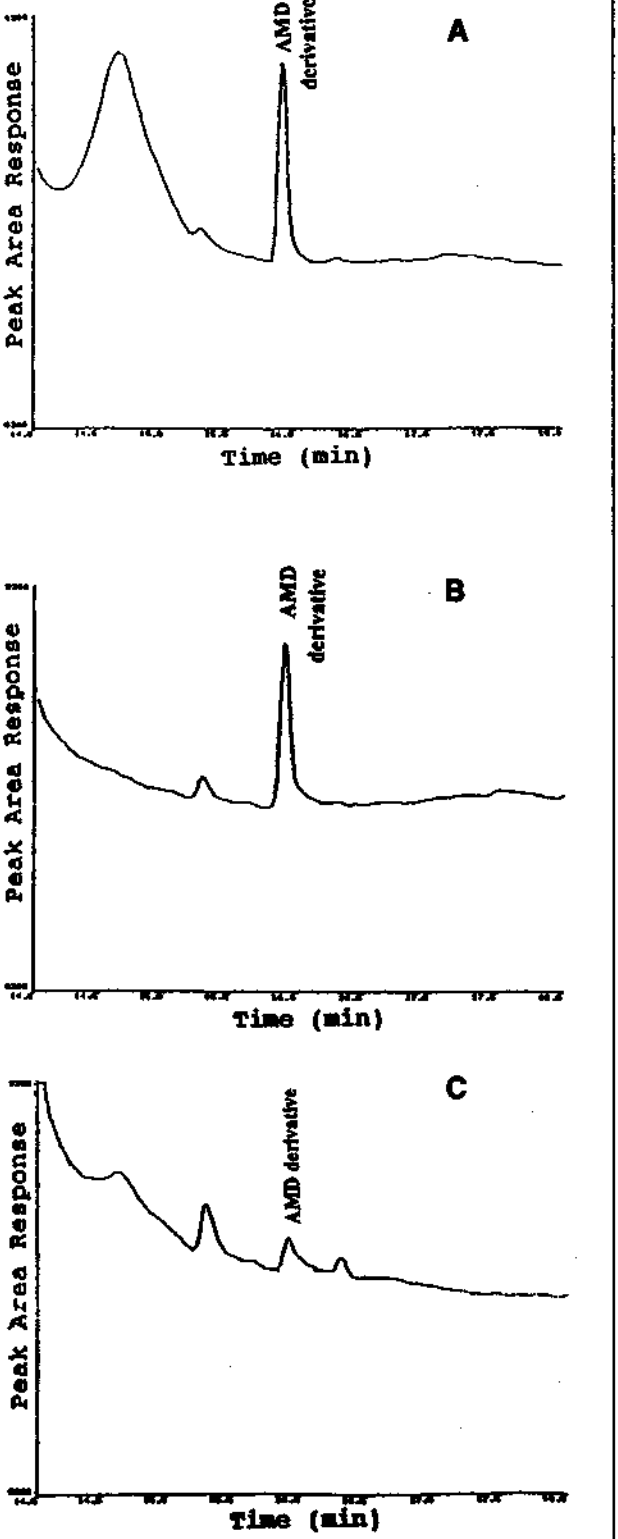

Figure 4. Chromatogram of corn extract blended for different periods: 10 s (A), 1 min (B), and 5 min (C). All samples were spiked with 50 ppb of AMD.

It is evident from this data that AMD was not present in any of the analyzed crops at any level above 10 ppb. The spiking data showed that AMD in soaked beans and fresh corn disappears much faster than in potatoes and sugar beets. The fresh corn and the soaked beans are more biologically active than the mature potatoes or sugar beets.

From the speed at which spiked AMD disappears, it is highly unlikely that AMD will be present at any level, even far below 10 ppb. For AMD to reach the final plant crop intact, it has to survive for a period of several months inside plant tissues while being subjected to different biological and chemical activities. Based on these results, as well as those provided by Castle et al. (4,5) and Arikawa and Minalo (6), the bioaccumulation of AMD in plant tissues is highly unlikely.

Previous studies have shown that AMD is not stable in soil

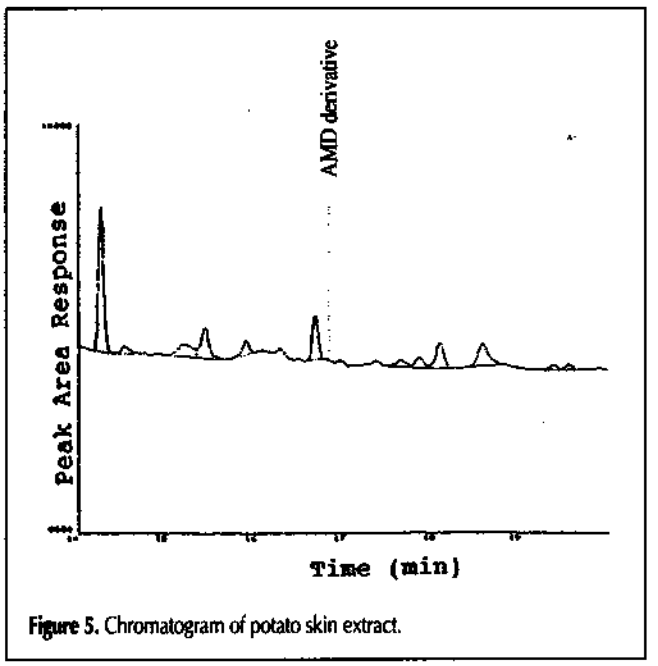

Figure 5. Chromatogram of potato skin extract.

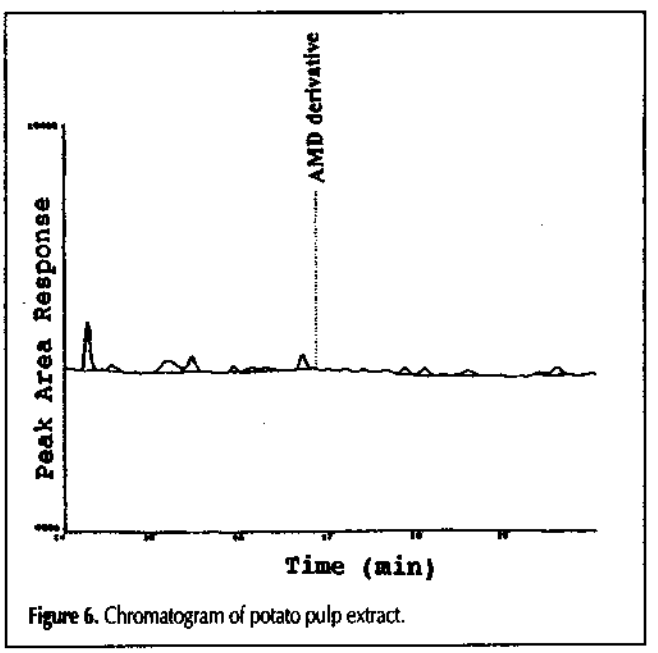

Figure 6. Chromatogram of potato pulp extract.

Table IV. Recovery of AMD from Potatoes

Blending time	Recovery from pulp (%)	Recovery from skin (%)
5 min	125	90
10 min	81	85
20 min	90	88

either. Lande et al. (12) showed that the half-life of AMD in aerobic soil is in the order of only several days at 20°C. The biotransformation of AMD in mammals and microorganisms (found naturally in soil) involves the irreversible conversion of AMD to acrylic acid. Shanker et al. (13) determined that AMD concentrations as high as 500 mg/kg in soil were reduced to undetectable levels in 5 days with the formation of acrylic acid as an intermediate. We found that under laboratory conditions and in HPLC-grade water, concentrations of AMD at 1000 ppm in glass flasks decreased by 50% in 4 weeks. This was basically due to the degrading effect of light. There was no significant loss of AMD in polyethylene flasks or when the glass flask was stored in the refrigerator. Naturally, under environmental conditions, the speed of hydrolysis will be much faster, as indicated by Lande et al. (12).

On the other hand, with regard to the fate of PAM, the consensus among most researchers is that PAM is a stable molecule. Although PAM will undergo some chemical reactions in the environment, it will not regenerate AMD monomer (1,14–16). Regeneration of the double-bond is not thermodynamically plausible. Thermal depolymerization does not occur; instead, other reactions take place upon heating PAM (e.g., loss of ammonia from the amide group) (1,16,17). PAMs are considered to have molecular sizes that are too large to be transported across biological membranes (3). The degradation of PAM in soil is basically due to mechanical degradation. It is also hydrolyzed at high pH or in the presence of biological compounds, microorganisms, or light.

Smith et al. (18,19) recently showed that subjecting PAM to certain conditions may cause an increase followed by a decrease in AMD concentration; the work is currently under evaluation. If PAM is depolymerized, the concentration of detected AMD should be significant in all cases. Also, the work assumed that the identity of a peak was AMD without using GC–MS to either verify AMD qualitatively or to disclaim it as chromatographic interference.

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